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A phylogenetic analysis of *Melanelia tominii* and four new records of brown parmelioid lichens from China

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Abstract –– The molecular analysis based on ITS nrDNA sequences indicates that *Melanelia tominii* probably belongs to *Melanelixia*. Four new records from China — *Melanelia predisjuncta*, *Melanohalea subelegantula*, *M. olivaceoides*, and *M. septentrionalis* — are reported. A key to the 21 species belonging to *Melanelixia*, *Melanohalea* and *Melanelia* from China is provided.

Keywords –– Asia, taxonomy, gyrophoric acid

Introduction

The lichen genus *Melanelia* (*Parmeliaceae*) was originally established by Esslinger in 1978. Two more genera, *Melanelixia* O. Blanco et al. and *Melanohalea* O. Blanco et al., were subsequently split from *Melanelia*, based on molecular as well as chemical and morphological data (Blanco et al. 2004). *Melanelixia* is characterized by having a pored or fenestrate epicortex, by lacking pseudocyphellae and by containing lecanoric acid as the primary medullary constituent (Blanco et al. 2004, Esslinger 1977). *Melanohalea* is characterized by pseudocyphellae, often on warts or isidial tips, by a non-pored epicortex, and by a medulla containing depsidones or lacking secondary compounds (Blanco et al. 2004, Esslinger 1977). The placement of the type species of *Melanelia*, *M. stygia* (L.) Essl., outside the parmelioid lichens was strongly supported in the molecular systematic studies (Blanco et al. 2004). Although *Melanelia tominii* resembles *M. stygia* morphologically, the former species contains the tridepside gyrophoric acid (usually with other tridepsides as well), and the latter contains

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the β-orcinol depsidones fumarprotocetraric and protocetraric acid (Esslinger 1977, 1992). Because molecular sequences were not available for *M. tominii*, its systematic position remained uncertain (Blanco et al. 2004).

Worldwide, *Melanelixia* includes nine known species, *Melanohalea* nineteen species and *Melanelia* still contains a heterogeneous residue of seventeen species (Esslinger 1977, 1978, 1987, 1992; Ahti et al. 1987, Egan 1987, Galloway & Jørgensen 1990, Thell 1995, Divakar et al. 2001, 2003; Blanco et al. 2004, Divakar & Upreti 2005, Wang et al. 2008). In China, *Melanelixia* includes seven species, *Melanohalea* five species and *Melanelia* five species (Wei 1991, Abbas & Wu 1998, Kurokawa & Lai 2001, Zibirnisa et al. 2004, Chen & Esslinger 2005, Wang et al. 2008).

During our study of these genera in China, four new records were discovered, namely *Melanelia predisjuncta*, *Melanohalea subelegantula*, *M. olivaceoides* and *M. septentrionalis*, and the systematic position of *M. tominii* was investigated based on its ITS sequences (including ITS1, 5.8S nrDNA and ITS2) and morphological and chemical characters. In addition, a key to 21 species belonging to *Melanelixia*, *Melanohalea* and *Melanelia* in China is provided.

Materials and methods

Morphology and Chemistry

The specimens studied are housed in HMAS-L (Lichen Section, Herbarium of the Institute of Microbiology, Academia Sinica) unless otherwise indicated. The morphology of the lichen specimens was examined using a Zeiss stereo microscope (Stemi SV 11) and Zeiss compound microscope (Axioscop 2 plus).

Table 1. Specimens of *Melanelia tominii* in which the morphology, chemistry or ITS sequences were studied.

Herbarium accession #	Specimen information	GenBank accession #
114000	CHINA. Hebei, Mt. Wulingshan, alt. 1750m, on rock, T. Zhang & H.Y. Wang, WLS‖042, May 17, 2004.	EU784154
036389	CHINA. Inner Mongolia, Mt. Arxan, alt. 1600m, on rock, J.C. Wei et al., Aer192, August 2, 2002.	EU784155
071058	CHINA. Inner Mongolia, Bairin Youqi, alt. 1800m, on rock, J.B. Chen & G.R. Hu, 21423, August 27, 2001.	EU784156
029902	CHINA. Sichuan, Mt. Gongga, alt. 3300m, on rock, X.Y. Wang et al., 8987, July 26, 1982	—
007086	CHINA. Hebei, Mt. Xiaowutaishan, alt. 2800m, on rock, J.C. Wei, 2042, August 16, 1964.	—
007087	CHINA. Tibet, Mt. Qomolangma, alt. 5000m, on rock, J.C. Wei & J.B. Chen, 1332, June 2, 1966.	—
077822	CHINA. Tibet, Chayu County, alt. 4250m, on rock, J.J. Su, 4801, September 26, 1982.	—
080967	U.S.A. Arizona, Cochise County, alt. 1830m, on rock, T.L. Esslinger, 12261, January 10, 1992.	—

Lichen substances in all specimens cited were identified using the standardized thin layer chromatography techniques (Culberson 1972). Information on the specimens of *M. tominii* studied is shown in Table 1.

Molecular systematics

Taxon sampling — Sequence data of the ITS nrDNA of *M. tominii* were obtained from three specimens (Table 1). Fifteen sequences of other related taxa were downloaded from GenBank (Table 2). *Lecanora leptyrodes* and *L. rupicola* were used as outgroup.

Table 2. Species and ITS sequences downloaded from GenBank.

Species	GenBank acc. #	Species	GenBank acc. #
Melanelia disjuncta	AY611077	*Melanohalea elegantula*	AY611094
M. hepatizon	AF451776	*M. exasperata*	AY611081
M. stygia	AY611121	*M. olivacea*	AY611091
		M. septentrionalis	AY611093
Melanelixia fuliginosa	AY611088	*M. subelegantula*	AY611115
M. glabra	AY611114	*M. subolivacea*	AY611123
M. subargentifera	AY611098	*Lecanora rupicola*	DQ451666
M. subaurifera	AY611099	*L. leptyrodes*	AY541255

PCR amplification and sequencing — Total DNA was extracted by the modified CTAB method (Rogers and Bendich 1988). DNA extracts were used for PCR amplification of the ITS nrDNA with ITS1 (White et al. 1990) and 1R (TATGCTTAAGTTCAGCGGGT) as primers. PCR reactions were performed in a DNA Thermal Cycler (Biometra) as follows: initial denaturation at 95°C for 3 min, followed by 35 cycles of 30 s denaturation at 94°C, 45 s annealing at 58°C, 1 min extension at 72°C, and completed with a final 8 min extension at 72°C. Products were purified with Gel Extraction Mini Kit (SABC). Sequencing reactions were carried out by Shanghai Genecore Corp. with an ABI 3700 Sequencer. Both complementary strands of each sample were sequenced.

Data analysis —The alignment was analyzed using the programs ClustalX 1.8.1. The aligned ITS matrix was edited manually and the flanking regions of the small subunit and large subunit rDNA were deleted through software MEGA 4 (Tamura et al. 2007). Phylogenetic analyses were conducted also in MEGA4. The phylogenetic tree was inferred using the Minimum Evolution method (Rzhetsky & Nei 1992), of which the reliability was tested by 1000 bootstrap replications (Felsenstein 1985). The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura et al. 2004). All positions containing alignment gaps and missing data were eliminated only in pairwise sequence comparisons (Pairwise deletion option).

The phylogenetic analysis of *Melanelia tominii*

Melanelia tominii (Oxner) Essl., Lichenologist 24(1): 17 (1992)
≡ *Parmelia tominii* Oxner, Zh. Bio.-Bot. Tsyklu, Kyev 1933(7–8): 171 (1933)
= *Parmelia substygia* Räsänen, Lichenes Fenniae Exs. 51 (1935)
≡ *Melanelia substygia* (Räsänen) Essl., Mycotaxon 7: 47 (1978)
= *Parmelia borisorum* Oxner, Bot. Zh., Kyyiv 1: 33 (1940)
= *Parmelia saximontana* R.A. Anderson & W.A. Weber, Bryologist 65: 236 (1963)
= *Parmelia altaica* Oxner, Ukr. bot. Zh. 27(2): 176 (1970)

ITS PHYLOGENETIC ANALYSIS — There were a total of 501 positions in the final dataset of ITS sequences. Six *Melanohalea* species, four *Melanelixia* species and four *Melanelia* species are included in the inferred tree based on ITS (FIG.1). All the *Melanohalea* species formed a monophyletic clade supported by 83% bootstrap value. All the *Melanelixia* species clustered in a clade supported by 70% bootstrap value. *Melanelia stygia* and *M. hepatizon* formed a monophyletic clade supported by 99% bootstrap value. The *Melanohalea* clade, the *Melanelixia* clade and *Melanelia disjuncta* form a large clade supported by 92% bootstrap value, while the clade comprised of *M. stygia* and *M. hepatizon* becomes the outgroup of the former three clades. Although *M. disjuncta* clustered in a clade together with *Melanohalea*, the clade has low bootstrap support (<50%) so the placement of *M. disjuncta* remains uncertain. These results are consistent with previous analyses based on polygenes (Blanco et al. 2004, Thell et al. 2002). In our study, *Melanelia tominii* 1 represents a specimen from Mt. Wuling, Hebei Province, China (herbarium accession no. 114000). *M. tominii* 2 represents two specimens from Inner Mongolia Province, China (herbarium accession nos. 036389, 071058), for which the ITS sequences are identical. *M. tominii* 1 and *M. tominii* 2 form a clade supported by 100% bootstrap value. Within the *Melanelixia* clade (70% bootstrap value), *M. tominii* and the interior clade comprised of *Melanelixia fuliginosa* and *M. subaurifera* (99% bootstrap value) form a moderately supported clade (58% bootstrap value), while another interior clade comprised of *M. glabra* and *M. subargentifera* (100% bootstrap value) becomes the outgroup of the three former clades. The fact that *M. tominii* locates within the *Melanelixia* clade in the phylogenetic tree, strongly indicates that *M. tominii* belongs to *Melanelixia* rather than *Melanelia*.

MORPHOLOGY AND CHEMISTRY — *Melanelia tominii* was included in the nominal subgenus *Melanelia* (Esslinger 1978) together with *M. stygia*, *M. disjuncta*, *M. panniformis*, *M. predisjuncta*, and *M. sorediata*. The rather small, dark, narrow lobed saxicolous thalli with the effigurate pseudocyphellae distinguish the members of this group from all the other species previously included in *Melanelia*. Further, both *M. stygia* and *M. tominii* have cylindrical to bifusiform conidia. However, *M. stygia* is chemically unique in this group by containing the β-orcinol depsidones (fumarprotocetraric and protocetraric

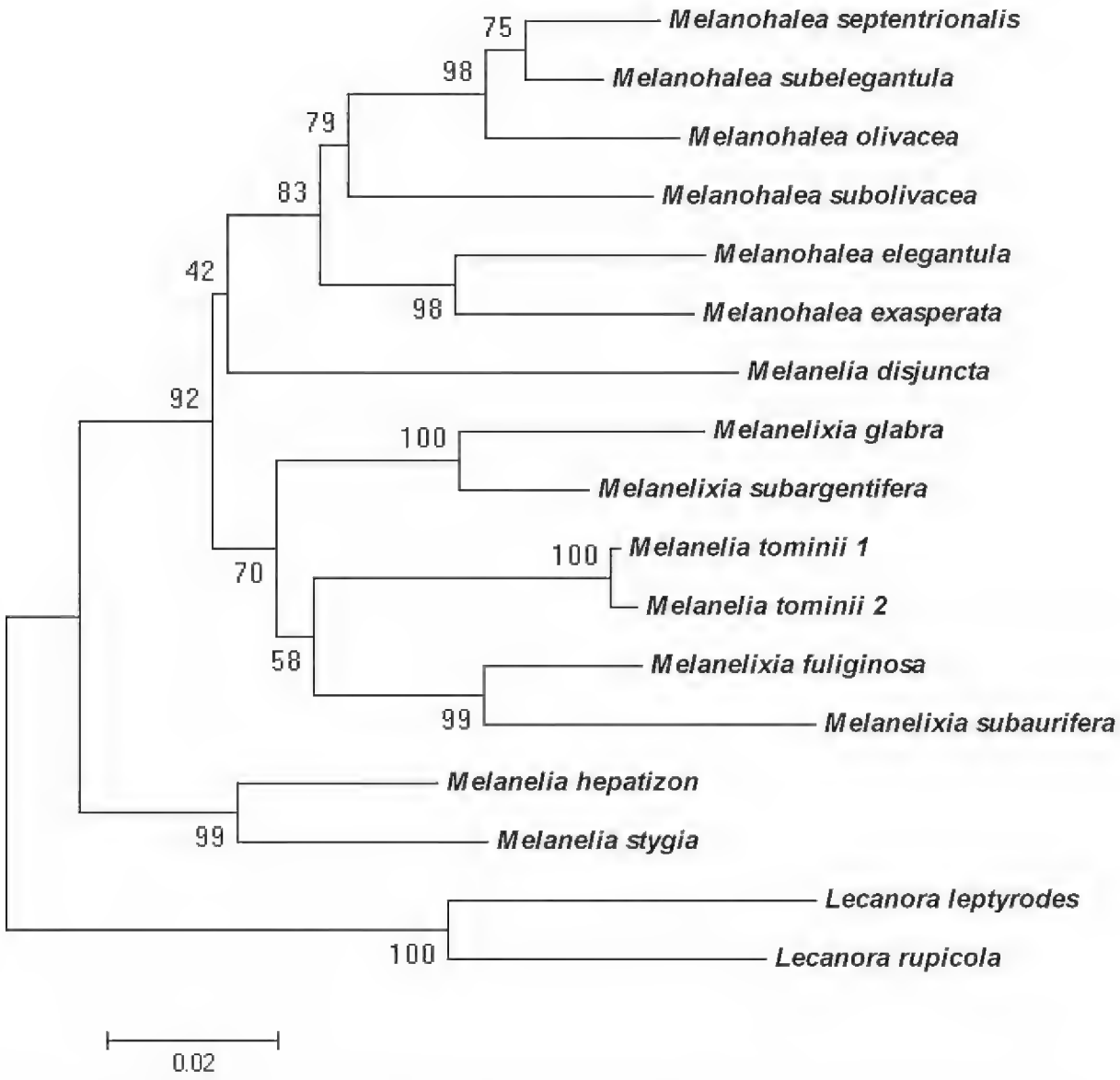

FIG. 1 The ME tree inferred from ITS data. The bootstrap values (1000 replicates) are shown next to the branches.

acids), while five of the other species contain orcinol *para*-depsides. *M. tominii* contains the tridepside gyrophoric acid, while *M. disjuncta*, *M. panniformis*, *M. predisjuncta*, and *M. sorediata* contain the depsides, perlatolic and stenosporic acids. The molecular analysis indicates that *M. disjuncta* and *M. tominii* are phylogenetically quite distant from *M. stygia*, but are closely related to *Melanelixia* and *Melanohalea* (FIG.1). *Melanohalea* species contain β-orcinol depsidones (six species contain fumarprotocetraric acid and one species norstictic acid) or lack lichen substances, while *Melanelixia* species contain the orcinol *para*-depside, lecanoric acid. Lecanoric and gyrophoric acids are closely related chemically as both derive from orsellinic acid moieties: the former is derived from two molecules of orsellinic acid, while the latter is derived from three. Thus *M. tominii* is also chemically similar to *Melanelixia*. In addition to *Melanelia tominii*, *M. microglabra*, *M. calva*, *M. fuscosorediata*,

M. glabratuloides, *M. piliferella*, *M. pseudoglabra*, and *M. subglabra* also produce gyrophoric acid as a major constituent (Esslinger 1977, Divakar et al. 2003). *M. tominii* is widely distributed in the northern hemisphere, and *M. microglabra* is known only from the type specimen in India, while the other six species are restricted to the Southern Hemisphere. As originally circumscribed (Esslinger 1978), the genus *Melanelia* comprised 46 species. The eight species with cortical hairs are now considered to belong to *Melanelixia* or the group containing gyrophoric acid, e.g. *M. fuscosorediata*, *M. piliferella*, and *M. pseudoglabra*. This evidence indicates that the species containing gyrophoric acid probably belong to *Melanelixia*, and the generic concept may be amended accordingly.

However, neither *M. tominii* nor the *Melanelia* species containing gyrophoric acid are transferred formally to *Melanelixia* in this paper, because of the paucity of our molecular data.

New records

1. ***Melanelia predisjuncta*** (Essl.) Essl., Mycotaxon 7(1): 47 (1978)
≡ *Parmelia predisjuncta* Essl., J. Hattori bot. Lab. 42: 50 (1977)

This species is characterized by the saxicolous habit, the narrow lobes (0.4–0.8 mm broad), the presence of pseudocyphellae, the lack of isidia and soredia, the acerose to slightly bifusiform conidia, the common apothecia, the black lower surface, the moderate rhizines, and the presence of perlatolic and stenosporic acids in the medulla (K–, C–, KC–, PD–). *M. predisjuncta* is superficially similar to *M. stygia*, but *M. stygia* can be readily distinguished by the more regular and distinctive pseudocyphellae, the bifusiform conidia, the thicker upper cortex (30–50 μm cf. 8–12 μm), and the absence of perlatolic and stenosporic acids. Of the three other species containing perlatolic and stenosporic acids, namely *M. disjuncta*, *M. sorediata* and *M. panniformis*, the former two have soralia, and the third species has distinctive laminal lobules. In our study only one specimen of *M. predisjuncta* was found. The specimen lacks apothecia, its upper cortex is 8–10 μm thick, lower cortex 9–12 μm thick and lobes 90–110 μm thick.

M. predisjuncta has been reported from Japan (Esslinger 1977) and Russia (Makryi 1981). New to China.

Specimen examined: CHINA. **Jilin**, Hongtoushan, Mt. Changbaishan, alt. 1900 m, on rock, J.C. Wei & J.B. Chen 6251 (HMAS-L: 052098).

2. ***Melanohalea subelegantula*** (Essl.) O. Blanco, A. Crespo, Divakar, Essl., D. Hawksw. & Lumbsch, Mycol. Res. 108(8): 883 (2004)
≡ *Parmelia subelegantula* Essl., J. Hattori bot. Lab. 42: 89 (1977)
≡ *Melanelia subelegantula* (Essl.) Essl., Mycotaxon 7(1): 48 (1978)

This species is characterized by the typically corticolous habit, the moderate lobes (1–3 mm broad), the lack of pseudocyphellae, soredia and pycnidia, the

rare apothecia, the black lower surface, the moderate rhizines, the absence of lichen substances (K–, C–, KC–, PD–), and the distinctive, small isidia (0.1–0.3 mm long). The isidia arise as hemispherical papillae, elongating into cylindrical isidia and then into lobules usually with rhizines and are unique in this genus. Among the five other species with isidia, *M. poeltii* contains fumarprotocetraric acid (PD + orange), *M. elegantula* has isidia with pseudocyphellae at the tips, *M. exasperatula* has hollow isidia, *M. infumata* has broader lobes [1–4(–6 mm)] and longer isidia (0.2–1 mm long), and *M. ushuaiensis* from southern South America has broader lobes (2–5 mm wide) and sparse isidia. In our study only one specimen of *M. subelegantula* was found. The specimen differs from typical *M. subelegantula* in the very dense cylindrical isidia, the very sparse lobules developing from isidia, and in the shorter rhizines (to 0.5 mm cf. to 1 mm long). The sparse lobules are short, small (60–110 × 40–100 µm), and occasionally rhizinate. The lobes of this specimen are 80–100 µm thick, upper cortex 8–12 µm thick, and lower cortex 8–12 µm thick.

Previously *M. subelegantula* was only known from western North America (Esslinger 1977). New to China.

Specimen examined: CHINA. **Tibet**, Gongbogyamda county, alt. 3500m, on bark, G.R. Hu h537, 24 Jul. 2004 (HMAS-L: 077825).

3. ***Melanohalea olivaceoides*** (Krog) O. Blanco, A. Crespo, Divakar, Essl., D. Hawksw. & Lumbsch, Mycol. Res. 108(8): 883 (2004)
≡ *Parmelia olivaceoides* Krog, Norsk Polarinst. Skr. 144: 109 (1968)
≡ *Melanelia olivaceoides* (Krog) Essl., Mycotaxon 7(1): 48 (1978)

This species is characterized by the typically corticolous habit, the moderate lobes (1–4 mm broad), the lack of pseudocyphellae, isidia and pycnidia, the laminal and punctiform soralia, the granular to isidioid soredia, the rare apothecia, the black lower surface, the moderate rhizines, and the presence of fumarprotocetraric and protocetraric acids (PD + orange, K–, C–, KC–, or sometimes lacking lichen substances). *M. olivaceoides* is related to the other five species containing fumarprotocetraric, namely *M. olivacea*, *M. septentrionalis, M. halei, M. poeltii*, and *M. gomukhensis*. However, the former four species are all esorediate, and *M. gomukhensis* has distinctive pseudocyphellae. In our study only one specimen of *M. olivaceoides* was found and this specimen lacked apothecia. The lobes were 80–100 µm thick, the upper cortex 8–12 µm thick and lower cortex 8–12 µm thick.

Melanelia olivaceoides has been reported from Alaska, Canada, United States, Europe, Siberia, Japan and Russia (Esslinger 1977, Makryi 1981). New to China.

Specimen examined: CHINA. **Tibet**, Gongbogyamda county, alt. 3500m, on bark, G.R. Hu h659, 24 Jul. 2004 (HMAS-L: 071066).

4. ***Melanohalea septentrionalis*** (Lynge) O. Blanco, A. Crespo, Divakar, Essl., D. Hawksw. & Lumbsch, Mycol. Res. 108(8): 883 (2004)

≡ *Parmelia olivacea* var. *septentrionalis* Lynge, Bergens Mus. Årbok 1912(10): 4 (1912).

≡ *Parmelia septentrionalis* (Lynge) Ahti, Acta Bot. Fenn. 70: 22 (1966)

≡ *Melanelia septentrionalis* (Lynge) Essl., Mycotaxon 7(1): 48 (1978)

This species is characterized by the corticolous habit, the shiny lobes (1–3 mm broad), the lack of isidia, soredia and lobules, the rare or absence of pseudocyphellae, the smooth apothecial margin, the subhymenium which is thinner than the hymenium, the acerose to slightly bifusiform conidia, the black lower surface, the moderate to dense rhizines, and the presence of fumarprotocetraric and protocetraric acid (PD+ orange, K–, C–, KC–). *M. septentrionalis* is closely related to *M. halei* and *M. olivacea*. However, *M. septentrionalis* can be separated from *M. halei* by the darker colored upper surface, the absence of papillae and lobules in the central parts of the thallus, the smaller spores (9–13 × 5.5–8.5 μm cf. 15–20 × 8–12.5 μm), the weakly bifusiform rather than cylindrical conidia, and the K– rather than K+ reaction of the medulla (yellow turning dingy orange for *M. halei*). *M. septentrionalis* differs from *M. olivacea* in its smaller thalli, the less common pseudocyphellae, the presence of numerous apothecia near the thallus periphery, and the smooth rather than crenate or tuberculate apothecial margin. In addition, *M. septentrionalis* is the only species of *Melanohalea* where the hymenium is obviously thicker than the subhymenium (2 × the thickness of the latter). In our study only one specimen of *M. septentrionalis* was found. The specimen had an obviously shiny and rugose upper surface. the lobes were 100–120 μm thick, the upper cortex 12–14 μm thick and lower cortex 10–12 μm thick. The hymenium was 70–100 μm high, and subhymenium 35–50 μm high. The spores are ellipsoid (9–11 × 6–8 μm), and spore wall 1 μm thick. Conidia are weakly bifusiform, 5–7 μm long.

M. septentrionalis has been reported from North America, Europe, Russia and India (Esslinger 1977, Öztürk 1990, Spribille & Kolb 2000, Motiejunaite 2002, Divakar & Upreti 2005). New to China.

Specimen examined: CHINA. **Heilongjiang**, Linchang, Mt. Dabaishan, alt. 1200 m, on bark, X.Q. Gao, 405, 3 Sep. 1984 (HMAS-L: 036135).

Key to *Melanelia*, *Melanelixia* and *Melanohalea* in China

1a. Thallus with soredia or isidia 2

1b. Thallus without soredia or isidia 12

2a. Thallus with isidia 3

2b. Thallus with soredia 9

3a. Medulla PD + red-orange, with fumarprotocetraric acid ..... *Melanohalea poeltii*

3b. Medulla PD –, other substances present or none 4

4a. Medulla C + rose or red 5
4b. Medulla C – 7
5a. Cortical hairs absent .....*Melanelixia fuliginosa*
5b. Cortical hairs obvious, especially on the lobe ends 6
6a. Isidia papillate, with obvious cortical hairs on the tips .....*Melanelixia villosella*
6b. Isidia cylindrical, without cortical hairs on the tips .....*Melanelixia subvillosella*
7a. Isidia mostly compressed-clavate to spathulate, hollow . *Melanohalea exasperatula*
7b. Isidia cylindrical, not hollow 8
8a. Pseudocyphellae present at the tip of isidia ..... *Melanohalea elegantula*
8b. Pseudocyphellae absent; some isidia growing into lobules with rhizines ..... *Melanohalea subelegantula*
9a. Medulla C + rose or red 10
9b. Medulla C –.....11
10a. Gyrophoric acid present, cortical hairs absent ..... *Melanelia tominii*
10b. Lecanoric acid present, cortical hairs present .....*Melanelixia subargentifera*
11a. Medulla P + red-orange, with fumarprotocetraric and protocetraric acid; corticolous; soralia punctiform, laminal .....*Melanohalea olivaceoides*
11b. Medulla P–, with perlatolic and stenosporic acid; saxicolous; soralia granular to isidioid, on the ends of the ascending lateral branches .....*Melanelia sorediata*
12a. Medulla P + orange or red-orange 13
12b. Medulla P – 16
13a. Pycnidia exogenous, medulla with stictic and norstictic acids ..... *Melanelia hepatizon*
13b. Pycnidia not exogenous, medulla with fumarprotocetraric and/or protocetraric acids 14
14a. Saxicolous; upper cortex thicker (30–50 μm); conidia bifusiform .....*Melanelia stygia*
14b. Corticolous; upper cortex thinner (8–14 μm); conidia acerose to weakly bifusiform 15
15a. Apothecia margin smooth; hymenium twice as thick as subhymenium .....*Melanohalea septentrionalis*
15b. Apothecia margin crenate or tuberculate; hymenium as thick as subhymenium ..... *Melanohalea olivacea*
16a. Medulla C + rose or red 17
16b. Medulla C– 20
17a. Medulla with gyrophoric acid; lobes distinctively pseudocyphellate; saxicolous ..... *Melanelia tominii*
17b. Medulla with lecanoric acid 18
18a. Cortical hairs present .....*Melanelixia glabra*
18b. Cortical hairs absent 19

19a. Thallus saxicolous; pseudocyphellae absent; apothecia rare ..... *Melanelixia glabroides*

19b. Thallus corticolous; pseudocyphellae present; apothecia common ..... *Melanelixia huei*

20a. Corticolous usually; no lichen substances detected; papillae conical, with conspicuous pseudocyphellae at the tip ..... *Melanohalea exasperata*

20b. Saxicolous; perlatolic and stenosporic acid present; without papillae 21

21a. Lobules abundant, marginal to laminal; pseudocyphellae absent or faint ..... *Melanelia panniformis*

21b. Lobules absent; pseudocyphellae obvious ..... *Melanelia predisjuncta*

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